Development of carbapenem resistance during therapy for non-typhoid *Salmonella* infection

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Abstract

Multidrug-resistant *Salmonella* infection is a global problem, and carbapenems may represent the last therapeutic choice. We report a case of infection caused by ceftriaxone-resistant and ciprofloxacin-resistant *Salmonella enterica* serotype Typhimurium. A bla_{CMY-2}-containing Tn*6092*, located on a self-transferable IncI1 plasmid, was found in all isolates derived from the patient. During ertapenem treatment, the strain developed carbapenem resistance. Apart from the OmpD deficiency found in all isolates, the strain further developed OmpC deficiency through a single gene mutation, and became carbapenem-resistant. *Salmonella* appears to be very plastic in developing antimicrobial resistance. Care must be taken by physicians when treating multidrug-resistant *Salmonella* infection.

Keywords: Carbapenem resistance, IncI1 plasmid, non-typhoid *Salmonella*, porin loss, Tn*6092*

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Non-typhoid *Salmonella* causes self-limited foodborne infections as well as invasive infections that require antimicrobial therapy [1]. Appropriate therapeutic agents may include ampicillin, trimethoprim–sulphamethoxazole, chloramphenicol, fluoroquinolones, or expanded-spectrum cephalosporins [2]. However, rates of resistance of *Salmonella* clinical isolates to the traditional antibiotics are high, and in recent years only fluoroquinolones and expanded-spectrum cephalosporins have remained effective against non-typhoid *Salmonella* infection [2]. Unfortunately, resistance to these agents has also increased in *Salmonella* [2]. For patients with invasive, multidrug-resistant *Salmonella* infection, carbapenems may represent the last resort [3]. The emergence of carbapenem resistance in *Salmonella* therefore represents a serious clinical problem, owing to the lack of other therapeutic choices.

In 2010, a clinical isolate of carbapenem-resistant *Salmonella enterica* serotype Typhimurium was identified in a 3500-bed university hospital in Taiwan. The study was undertaken to investigate the associated carbapenem resistance mechanisms. Antimicrobial susceptibility of the isolates was examined by a standard disk diffusion method, and MICs were assessed with Etest strips (AB Biodisk, Solna, Sweden). The results were categorized according to the suggestions of the CLSI [4]. The genetic relatedness of the isolates was investigated by pulsed-field gel electrophoresis [5]. Genes for cephalosporinases (SHVs, TEMs, CTX-Ms, and AmpCs) and carbapenemases (IMPs, KPCs, VIMs, and OXAs) were amplified by PCR and sequenced [6–8]. Plasmid analysis and DNA–DNA hybridization were used to locate the resistance genes [6]. Conjugation, replicon typing and plasmid multilocus sequence typing were used to characterize the resistance plasmids [9,10]. The expression of the two major outer membrane proteins, OmpC and OmpD, of *Salmonella* Typhimurium was analysed by RT-PCR with the following primer pairs designed according to the published sequence (GenBank accession no. FQ312003): RT-OmpC-F, 5′-GGC GCTATCACCACGTCTAAAC-3′; RT-OmpC-R, 5′-TTGG CAAAACCGTAAGAGG-3′; RT-OmpD-F, 5′-GCGCAGT ACCAGGGCAAAAACGAC-3′; and RT-OmpD-R, 5′-CAGA CCAGCAGCCCAGACTTCAGC-3′. DNA sequences of the two outer membrane proteins were also analysed by PCR and sequencing with the following primer pairs (GenBank accession no. FQ312003): OmpC-F (5′-ATCCGGTTGAAA TAGGGGTAA-3′) and OmpC-R (5′-GGTGGACATGTTT TTGTTGAAGTA-3′); and OmpD-F (5′-GTGCTCCYCCTGC GCCATACCA-3′) and OmpD-R (5′-TCCGCAAAGACAAC GACCAGTGAA-3′).

A 77-year-old woman with nephritic syndrome and chronic renal insufficiency was hospitalized several times for medical treatment. On 14 February, she had an onset of urinary tract infection, and was hospitalized again through

the emergency department. *Salmonella* Typhimurium (U1) was identified in the urine culture. The strain was resistant to ampicillin, chloramphenicol, trimethoprim–sulphamethoxazole, and ceftriaxone, but remained susceptible to carbapenems (ertapenem, 0.064 mg/L; imipenem, 0.25 mg/L) and tigecycline. Ciprofloxacin was reported as resistant, with a resistant zone diameter (14 mm) of nalidixic acid according to the CLSI's suggestions [4]. The patient was given ertapenem (1 g once-daily), but developed diarrhoea during the therapy. Stool culture yielded *Salmonella* Typhimurium (S1) of the same antibiogram. Follow-up cultures of both urine and stool specimens invariably grew *Salmonella* Typhimurium (U2/S2 and U3/S3), also of the same antibiogram. The patient was discharged after the symptoms and signs of urinary tract infection resolved. In April, the patient was hospitalized again, owing to a right leg crush injury. The initial wound culture yielded ceftriaxone-resistant *Proteus mirabilis*, and ertapenem was prescribed on 19 April. She received wound debridement on 21 April. The wound pus yielded *Salmonella* Typhimurium (W1) of the same antibiogram as those described above (Table 1). The patient subsequently had persistent diarrhoea, and the stool culture again grew *Salmonella* Typhimurium (S4). The antibiogram of strain S4 was similar to those of the other *Salmonella* Typhimurium isolates derived from this patient, except that strain S4 was resistant to carbapenems (ertapenem, >32 mg/L; imipenem, 8 mg/L) (Table 1). The patient was put under contact isolation, and was kept on ertapenem therapy. She was discharged without serious sequelae after appropriate wound debridement.

A total of eight isolates, three from urine, four from stool, and one from wound cultures, of *Salmonella* Typhimurium were recovered from the patient. Except for strain U2, the remaining seven isolates were available for further molecular investigation (Table 1). Identical pulsed-field gel electrophoresis patterns were found among the isolates, indicating that the isolates were of the same lineage. A bla_{CMY-2} gene was present in all isolates, and was the only ceftriaxone resistance gene identified. The bla_{CMY-2} gene was located in a Tn*6092* that has previously been identified in *S. enterica* serotype Choleraesuis SC-B67 and several members of the *Enterobacteriaceae* [6,11]. The Tn*6092* was carried on a 115-kb IncI1 plasmid of the ST54 plasmid multilocus sequence type. The IncI1 plasmid was demonstrated to be self-transferable. MICs of ceftriaxone increased from 0.064 to 64 mg/L, whereas those of carbapenems (ertapenem, 0.064 mg/L; imipenem, 0.25 mg/L) remained low, in the recipient strain *Escherichia coli* J53 after receiving the bla_{CMY-2}-containing IncI1 plasmid.

None of the known carbapenemase genes was identified from the carbapenem-resistant strain, S4. The low MIC (1 mg/L) of tigecycline suggested that efflux pumps were not

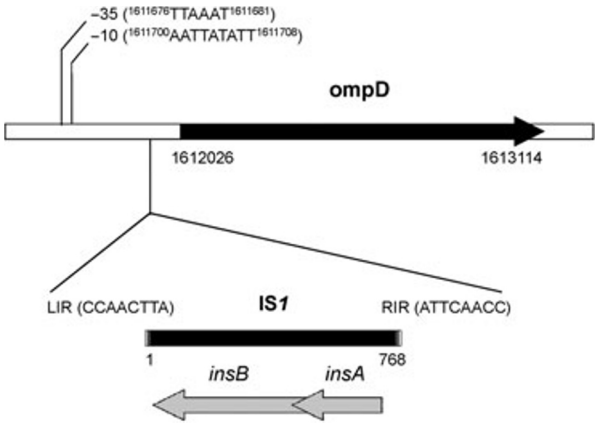

FIG. 1. Schematic presentation of *ompD* (*Salmonella* Typhimurium SL1344, FQ312003) genetic structure, showing the insertion of IS*1* in the carbapenem-resistant *Salmonella* Typhimurium (S4) isolate.

TABLE 1. Laboratory finding among the *Salmonella* Typhimurium isolates studied

	Specimen			RNA expression[b]		
Bacteria	Date	Type	Plasmid profile[a] (kb)	OmpD[c]	OmpC[d]	OmpC[d], amino acid 77
U1	14 February	Urine	(115, 210)	0.0006	1.09	W (TGG)
S1	19 February	Stool	(115)	0.0008	0.88	W (TGG)
S2	2 March	Stool	(115, 210)	0.0007	0.98	W (TGG)
S3	9 March	Stool	(115, 210)	0.0008	1.14	W (TGG)
U3	9 March	Urine	(115, 210)	0.0011	0.61	W (TGG)
W1	21 April	Wound	(115, 210)	0.0004	0.97	W (TGG)
S4	27 April	Stool	(115, 210)	0.0056	0.04	Stop (TGA)

[a]The bla_{CMY-2}-harbouring IncI1 plasmid is underlined.
[b]RNA expression level is given as the fold of expression relative to that of 16S rRNA in each strain as revealed by RT-PCR.
[c]As compared with that of *Salmonella* Typhimurium LBNP4417, the expression level of OmpD was low among the isolates, presumably because of an IS*1* insertion between *ompD* and its promoter region (Fig. 1).
[d]As compared with that of other isolates tested, the expression level of OmpC was low in the carbapenem-resistant strain, S4, probably because of the amino acid change from tryptophan (W, TGG) to a stop codon (TGA) at codon 77.

involved in the carbapenem resistance. OmpD deficiency was found in all isolates, probably because of the insertion of IS*1* 88 bp upstream of *ompD* (Fig. 1). In the carbapenem-resistant strain, S4, OmpC deficiency was also evident, as shown by the low expression level revealed by RT-PCR (Table 1). A point mutation was identified at codon 77 of ompC, leading to an amino acid change from tryptophan (TGG) to a stop codon (TGA), and thus the early termination of the protein (Table 1).

Carbapenem resistance resulting from the production of extended-spectrum β-lactamases (ESBLs) or AmpCs plus porin deficiency is frequently reported in *E. coli* and *Klebsiella pneumoniae* [7,8], but very rarely in *Salmonella*. There has been only one report demonstrating, in an imipenem-resistant strain of *S. enterica* serotype Wien, that the imipenem resistance was related to CMY-4 β-lactamase production and porin loss [12]. In the present study, OmpD, an analogue of OmpK35 in *K. pneumoniae* or OmpF in *E. coli*, was deficient in every isolate. This is common for isolates that produce ESBLs or AmpCs [7,8]. After the use of ertapenem, the expression of OmpC, an analogue of OmpK36 in *K. pneumoniae* or OmpC in *E. coli*, was further reduced, leading to the carbapenem resistance phenotype.

Carbapenem resistance can also occur through the production of carbapenem-hydrolysing enzymes [13]. Although the mechanism is frequently reported in *E. coli* and *K. pneumoniae* [13], the presence of such enzymes, including IMP-4, IMP-13, and KPC-2, has been only sporadically reported in *Salmonella* [14–16]. Carbapenem resistance resulting from the production of carbapenemases in *Enterobacteriaceae* is infrequent in Taiwan [7,8]; in contrast, another mechanism associated with the production of ESBLs or AmpCs and porin deficiency appears more commonly.

We previously identified, in several serotypes of non-typhoid *Salmonella*, a self-transferable bla_{CMY-2}-harbouring IncI1 plasmid that has contributed to the overall increase of ceftriaxone resistance in *Salmonella* [10]. In the case reported herein, the ceftriaxone resistance of all *Salmonella* Typhimurium isolates was also attributable to the presence of such self-transferable IncI1 plasmids. The porin change occurred 8 days after the use of ertapenem during the second hospitalization of the patient, and resulted in a more than 32-fold increase in the carbapenem MICs.

Increasing ceftriaxone resistance caused by the spread of ESBL or AmpC genes in non-typhoid *Salmonella* has been reported worldwide [10,17–19]. Our study has demonstrated that resistance of these ceftriaxone-resistant *Salmonella* isolates to carbapenems may emerge during ertapenem therapy. *Salmonella* appears to be very adaptive to antimicrobial selection pressure [20]. Physicians should be aware of this possibility, and all patients under therapy for non-typhoid *Salmonella* infection should be carefully monitored.

Transparency Declaration

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